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Ring-closing alkyne metathesis mediated synthesis of cyclic β-turn mimetics

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Abstract—The application of ring-closing alkyne metathesis to synthesise conformationally restricted peptidic β-turn mimics has been investigated. A range of oligopeptides containing either two acetylenic amino acids, or two cysteine residues have been synthesised and subjected to suitable cyclisation conditions. The structures of the cyclic compounds are investigated by 2D NMR analysis.

Reverse turns are important structural elements contributing to the folding and functioning of peptides and proteins.[1] The β-turn consists of four consecutive residues in a peptide chain, with the C=O of the first residue (*i*) H-bonded to the NH of the fourth residue $(i+3)$. In nature, several factors can be responsible for the formation of β-turns. Peptides having specific amino acid residues at $(i+1)$ and $(i+2)$—for instance the combinations Gly and Asn/Asp, Pro and Phe/Gly/Asn/Tyr—are often found to adopt a β-turn conformation. Alternatively, proteins featuring a Cys-AA-AA-Cys tetrapeptide sequence may form a β-turn type structure through the formation of a disulfide bridge.[2] This is exemplified by the active sites of the enzymes glutaredoxin and thioredoxin reductase, both of which contain cystine-containing tetrapeptides, Cys-Pro-Tyr-Cys and Cys-Ala-Thr-Cys, respectively.[3]

In recent years the design of synthetic, non-natural turn motifs that enable the introduction of conformational constraints in peptide-based materials has received wide interest.[4] Installment of a β-turn can be achieved through the introduction of a properly designed dipeptide isostere at positions $(i+2)$–$(i+3)$ of the target oligopeptide. For instance, we and others, have reported on the design and synthesis of sugar amino acids (SAAs),[5] that is carbohydrate derivatives having an amine and a carboxylic acid for incorporation in oligopeptide sequences. An alternative strategy towards synthetic β-turns entails the replacement of disulfide bridges in cystine-containing peptides by non-natural functionalities. With the aim to attain metabolically stable peptides, several groups have successfully demonstrated the ring-closing metathesis (RCM)-mediated cyclisation of oligopeptides containing two allylglycine residues.[6] Reduction of the ensuing mixture of (*Z*)- and (*E*)-alkenes affords cyclic peptides featuring a cystine mimetic, of which the disulfide moiety is replaced by a conformationally more flexible ethane bridge (i.e., (2*S*, 7*S*)-diaminosuberic acid).

We recently disclosed that application of ring-closing alkyne metathesis (RCAM)[7] of acetylenic amino acid-containing templates enables the preparation of cystine isosters with a rigid acetylene moiety replacing the natural disulfide bridge.[8] In line with these studies, we here report the RCAM-mediated cyclisation of oligopeptides containing two (*S*)-2-amino-4-hexynoic acid derivatives.

We further present the structural analysis, through 2D NMR measurements, of two of the resulting cyclic

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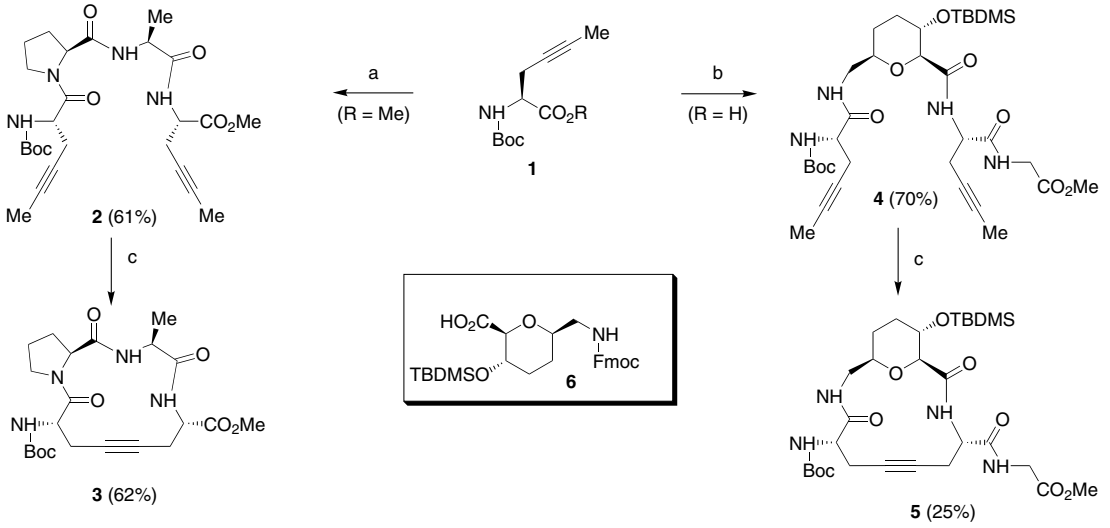
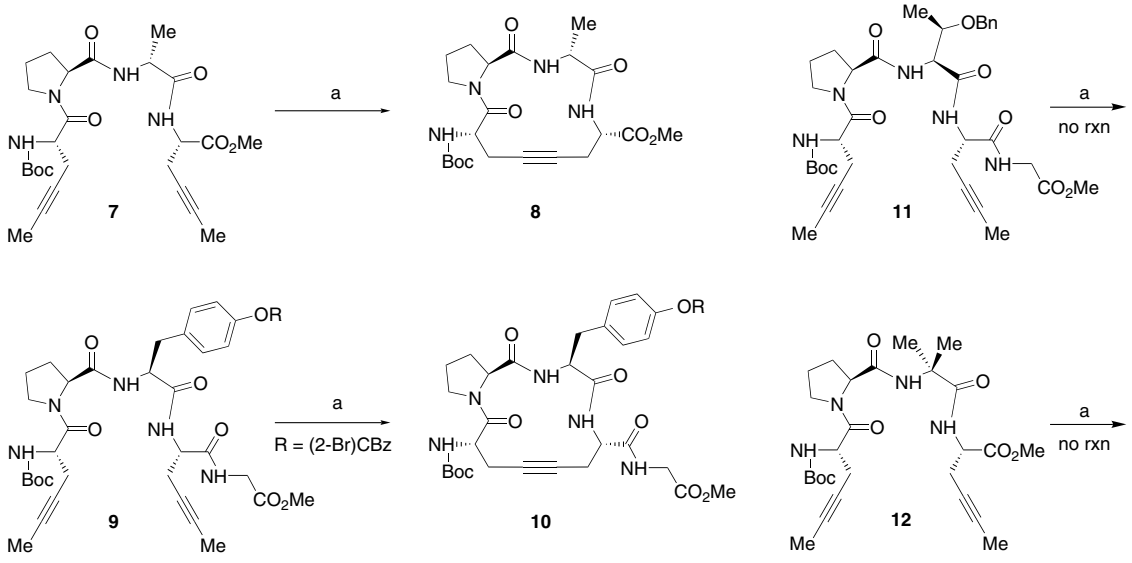
peptides, that contain either a pyranoid SAA dipeptide isostere (**5**, Scheme 1) or the Tyr-Pro dipeptide (**10**, Scheme 2) at positions $(i+1)$–$(i+2)$, in comparison with their cystine-containing counterparts (**14** and **15**, Figure 1).

Our synthetic strategy is exemplified by the synthesis of cyclic tetrapeptides **3** and **5** (Scheme 1). (*S*)-2-(*tert*-butoxycarbonylamino)-4-hexynoic acid methyl ester **1** (R = Me), prepared according to the literature procedure,[9] was treated with trifluoroacetic acid in the presence of triisopropylsilane (TIS). Condensation of the resulting free amine with Boc-Ala-OH in the presence of Castro's reagent and NMM in DMF followed by solution phase peptide synthesis-mediated elongation using the appropriate Boc-protected amino acid building blocks gave fully protected tetrapeptide **2** in 61% yield. RCAM of **2** using the tungsten catalyst $(^t\mathrm{BuO})_3\mathrm{W}{\equiv}\mathrm{C}^t\mathrm{Bu}$ (9 mol%, chlorobenzene or toluene, 80 °C, 3 h)[10] gave the desired cyclic tetrapeptide **3**[11] in 62% yield after silica gel flash chromatography. In an alternative synthetic sequence, (*S*)-2-(*tert*-butoxycarbonylamino)-4-hexynoic acid **1** (R = H) was condensed with glycine methyl ester (BOP, NMM, DMF). After removal of the Boc protective group (TFA, TIS, CH_2Cl_2) the resulting dipeptide was condensed with SAA building block **6**, followed by removal of the Fmoc protective group (20% piperidine in DMF) and final condensation with **1** (R = H) to afford tetrapeptide **4**[12] in 70% yield. RCAM of **4** under the aforementioned con-



Scheme 1. Reagents and conditions: (a) (i): TFA/CH_2Cl_2/TIS 1/1/0.01, (ii): Boc-Ala-OH, BOP, NMM, DMF, (iii): TFA/CH_2Cl_2/TIS 1/1/0.01, (iv): Boc–Pro–OH, BOP, NMM, DMF, (v): TFA/CH_2Cl_2/TIS 1/1/0.01, (vi): **1** (R = H), BOP, NMM, DMF. (b) (i): glycine methyl ester, BOP, NMM, DMF, (ii): TFA/CH_2Cl_2/TIS 1/1/0.01, (iii): **6**, BOP, NMM, DMF, (iv): 20% piperidine/DMF, (v): **1** (R = H), BOP, DiPEA, DMF. (c) $(^t\mathrm{BuO})_3\mathrm{W}{\equiv}\mathrm{C}^t\mathrm{Bu}$ (9 mol%,) chlorobenzene, 80 °C.



Scheme 2. Reagents and conditions: (a) $(^t\mathrm{BuO})_3\mathrm{W}{\equiv}\mathrm{C}^t\mathrm{Bu}$ (9 mol%,) chlorobenzene, 80 °C.

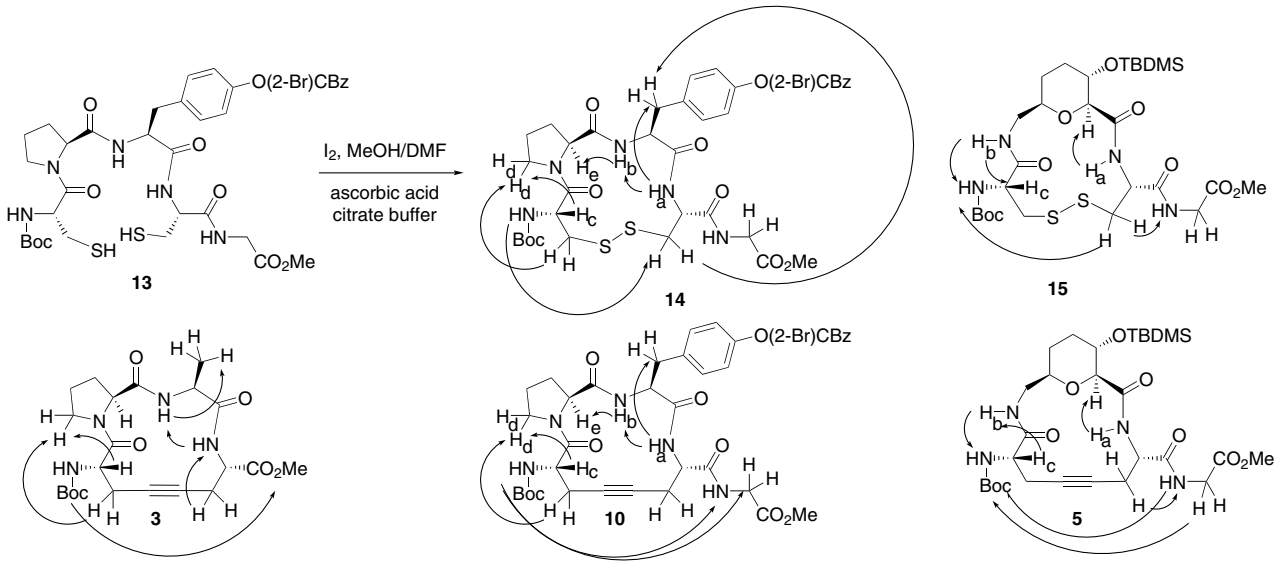


Figure 1. Proton–proton interactions determined from the NOESY spectra of peptides **3**, **5**, **10**, **14** and **15**, recorded in $CDCl_3$.

ditions followed by silica gel purification gave the fully protected cyclic tetrapeptide **5**[13] in a moderate yield (25%).

The scope of the RCAM-mediated cyclisation was further evaluated using oligopeptidic diynes **7**, **9**, **11** and **12** (Scheme 2; these oligopeptides were readily prepared using standard solution phase peptide synthesis, as outlined above). RCAM of **7**, the epimer of **2** containing (*R*)-alanine instead of (*S*)-alanine, proceeded uneventfully to afford the cyclic peptide **8** (70% yield). Similarly, the linear pentapeptide **9** was transformed into the cyclic pentapeptide **10**,[14] albeit in only 36% yield. In contrast, cyclisation of **11** and **12**, with Thr-Ala and Aib-Ala linking the two acetylenic amino acid derivatives, proved to be abortive. These results indicate that the nature of the dipeptide (mimic) linking the two acetylenic amino acid moieties plays a crucial role for an ensuing productive RCAM event.

At this stage, compounds **5** and **10** were analysed by NMR and compared with their cystine-containing counterparts **14** and **15** (Figure 1). The precursors of the latter cyclic peptides were readily prepared following peptide synthesis protocols as outlined for the synthesis of the linear peptides, but employing Boc-Cys-(SAcm)-OH instead of 2-amino-4-hexynoic acid derivative **1**. Oxidation of the cysteine residues was readily accomplished under standard conditions (I_2, MeOH, DMF, ascorbic acid, citrate buffer) to afford the target cyclic peptides **14** and **15** in 55% and 61% overall yields, respectively. Relevant NOE signals observed for peptides **5**, **10**, **14** and **15** are depicted in Figure 1. Comparison of the NOESY spectra of **10** and **14** revealed several similarities. In both cases H_c/H_d interactions were present, indicating a *trans*-amide bond at this side of the molecules. Furthermore, NOEs between H_a/H_b and H_b/H_e suggest a β-turn as the major structural motif for both protected peptides. The most remarkable difference entails the presence of NOE signals between NH-Boc and the NH- and α-protons of the glycine residue in **10**, all of which are absent in cystine analogue **14**. Presumably, the rigid acetylene bridge forces these protons to be in close proximity. Attempts to study the involvement of amide protons in H-bonding through either temperature-dependent chemical shift studies (spectra were recorded in DMSO-d_6) or solvent titration studies were abortive for both **10** and **14** due to overlap of the amide signals and the aromatic protons. Comparison of the NOESY spectra of **5** and **15** revealed similar patterns to those observed for **10** and **14**. Again, NOE's between NH-Boc and the glycine NH- and α-protons were present for the acetylene derivative **5**, but not for the cystine analogue **15**. Temperature dependent chemical shift studies (DMSO-d_6) of cyclic peptide **5** revealed a $\Delta\delta/\Delta t$ value for NH_a of −0.003 ppm/K, indicating that it may be involved in a H bond, presumably with the carbonyl of residue *i* as is the case in a regular β-turn. The values for the other amide protons in **5**, as well as for those in **15** were higher than −0.003 ppm/K.

In conclusion, we have demonstrated for the first time that RCAM can be applied in the synthesis of dicarba analogues of cystine-containing cyclic peptides. Our strategy allows the introduction of geometrically pure acetylenic cystine isosters in oligopeptide sequences, although in order to allow general application, the efficiency of the transformation requires future improvement. Preliminary structural analysis indicates that replacement of a disulfide bridge with an acetylene moiety renders the cyclic peptides more rigid, favouring additional interstrand proton–proton interactions.

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11. The reaction was performed under an Ar atmosphere in flame-dried glassware. To a solution of (^{t}BuO)$_3$W≡C^{t}Bu (10 mg, 0.02 mmol) in dry C_6H_5Cl (5.5 mL) was added a solution of **2** (200 mg, 0.23 mmol) in dry C_6H_5Cl (5.5 mL) and the reaction mixture was stirred at 80 °C for 3 h. The solvent was concentrated and the crude mixture was purified by column chromatography (EtOAc) to give **3** in 62% yield. ^{1}H NMR (400 MHz, CDCl3) 7.63 (bd, 1H), 6.58 (bd, 1H), 5.80 (bd, 1H), 4.81 (m, 1H), 4.60 (m, 1H), 4.31 (m, 1H), 4.17 (m, 1H), 3.82 (s, 3H), 3.75–3.62 (m, 1H), 3.61–3.56 (m, 1H), 2.71–2.62 (m, 4H), 2.24–1.90 (m, 4H), 1.53 (d, 3H, $J = 7.1$ Hz), 1.44 (s, 9H). ^{13}C NMR (75 MHz, CDCl3) δ 170.5, 170.3, 170.1, 169.6, 154.3, 79.6, 78.2, 76.8, 61.7, 52.4, 50.9, 50.6, 50.2, 47.3, 29.5, 28.4, 28.1, 25.5, 22.6, 22.5. IR ν 3311, 2926, 1745, 1643, 1504, 1444, 1368, 1163, 1061, 1030, 911, 734 cm^{-1}. (FAB) m/z calcd for $C_{22}H_{32}N_4O_7Na$ $[M + Na]^+$ 487.21687 found 487.21656.
12. Glycine methyl ester hydrochloride was dissolved in DMF (10 mL/mmol) and (*S*)-2-(*tert*-butoxycarbonylamino)-4-hexynoic acid (**1**, 1.5 equiv) was added followed by BOP (1.5 equiv) and NMM or DIPEA (4 equiv). The reaction mixture was stirred under N_2 at room temperature for 4–8 h. After the reaction was complete (TLC), the solvent was evaporated and the residue redissolved in CH_2Cl_2, washed with aqueous saturated $NaHCO_3$, water, $KHSO_4$ (0.05 M) and brine, dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography on silica gel (Pet. Ether–EtOAc 2:1).The Boc protecting group was removed by treatment with a solution of TFA/CH_2Cl_2/TIS 1/1/0.01 (10 mL/mmol) for 15 min at room temperature and subsequent evaporation to dryness coevaporating with toluene. Sugar amino-acid building block **6** was then coupled under the same conditions as just described (BOP, DiPEA, DMF; column chromatography: Pet. Ether–EtOAc 3:1 → EtOAc). The Fmoc protecting group was removed using a solution of piperidine in DMF (20%, 8 mL/mmol). After stirring for 15 min at room temperature, the mixture was evaporated to dryness and coevaporated twice with DMF to afford the corresponding tripeptide with the free terminal amino group which was reacted with (*S*)-2-(*tert*-butoxycarbonylamino)-4-hexynoic acid (**1**) under the described coupling conditions (BOP, DiPEA, DMF; column chromatography:Pet.Ether–EtOAc 1:1 → EtOAc) to give **4** in 70% yield. $[\alpha]_D^{20}$ −24.0 ($c = 0.1$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09–6.82 (3 × bs, each 3H), 5.49 (bs, 1H), 4.51 (m, 1H), 4.24 (m, 1H), 4.08 (m, 2H), 3.79 (s, 3H), 3.68–3.49 (m, 4H), 3.23 (m, 1H), 2.80–2.52 (m, 2H), 2.07 (m, 1H), 1.80 (s, 3H), 1.76 (s, 3H), 1.71–1.50 (m, 3H),1.43 (s, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 170.1, 169.9, 155.3, 82.1, 79.9, 79.2, 76.1, 73.9, 69.2, 53.4, 52.3, 51.5, 43.2, 41.2, 32.7, 28.1, 27.1, 25.5, 22.7, 22.1, 3.3, −4.8, −5.5. HRMS m/z: calcd for $C_{33}H_{55}N_4O_9Si$ $[M + H]^+$: 679.3738; found 679.3766.
13. Cyclic tetrapeptide **5**: $[\alpha]_D^{20}$ −3.1 ($c = 0.1$, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.51(d, 1H), 7.49 (bs, 1H), 6.82 (bs, 1H), 6.21 (d, 1H), 4.60 (m, 1H), 4.45 (m, 1H), 4.12 (m, 1H), 3.98 (m, 1H), 3.78 (m, 2H), 3.76 (s, 3H), 3.63 (m, 2H), 3.38 (m, 1H), 2.92 (m, 1H), 2.81 (m, 1H), 2.60 (m, 1H), 2.55 (m, 1H), 1.99 (m, 1H), 1.69–1.55 (m, 3H), 1.45 (s, 9H), 0.87 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.8, 79.5, 75.2, 69.1, 52.9, 52.5, 49.6, 43.0, 41.5, 29.7, 28.3, 26.6, 25.7, 23.1, 23.0, −4.8, −4.9. HRMS m/z: calcd for $C_{29}H_{49}N_4O_9Si$ $[M + H]^+$: 625.3268; found 625.3220.
14. Cyclic pentapeptide **10**: $[\alpha]_D^{20}$ −2.6 ($c = 0.3$, CH_2Cl_2). ^{1}H NMR: δ 8.15 (bs, 1H), 7.89 (d, 1H, $J = 7.9$ Hz), 7.61–7.06 (m, 8H), 6.54 (d, 1H, $J = 7.0$ Hz), 5.98 (d, 1H, $J = 8.3$ Hz), 5.35 (s, 2H), 4.63 (bs, 1H), 4.55 (bs, 1H), 4.29 (bs, 1H), 4.16 (t, 1H, $J = 7.5$ Hz), 4.10 (m, 1H), 3.91 (m, 1H), 3.74 (s, 3H), 3.72–3.53 (m, 3H), 3.32 (m, 1H), 2.73–2.59 (m, 4H), 2.05 (m, 2H), 1.95 (m, 1H), 1.84 (m, 1H), 1.40 (s, 9H). ^{13}C NMR: δ 172.3, 171.6, 170.9, 170.7, 170.4, 155.4, 155.3, 135.2, 132.8, 129.9, 127.5, 123.3, 121.5, 121.0, 80.0, 78.8, 77.2 (C≡), 69.5, 62.0, 56.8, 52.1, 51.7, 50.5, 47.1, 41.1, 34.6, 28.6, 28.1, 25.5, 22.8, 21.7. HRMS m/z: calcd for $C_{38}H_{45}BrN_5O_{11}$ $[M + H]^+$: 826.2298; found 826.2257.